Predation on the California Sea Hare, *Aplysia californica* Cooper, by the Solitary Great Green Sea Anemone, *Anthopleura xanthogrammica* (Brandt), and the Effect of Sea Hare Toxin and Acetylcholine on Anemone Muscle

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Because there are no known predators to feed on them in their adult state, the sea hares do not seem to enter into the prey-predator relationships of the sea. They do, however, appear to have a place in the food economy in certain limited ways. Great numbers of larvae are produced by the sea hares (MacGinitie, 1934), which presumably are consumed in large numbers by predaceous plankton and filter-feeders. Large quantities of sea weed are masticated, partially digested, and passed in the fecal pellets, thus somewhat abbreviating the process by which sea weed becomes detritus. Finally, when the adults die their bodies become a part of the marine economy by providing nutrition for bacterial flora, or perhaps for scavengers such as *Pachygrapsus,* which on occasion have been observed feeding on the bodies of dead sea hares.

Fitch of the California Fish and Game Laboratories (1961) virtually eliminates predation by fish; he and his staff have examined the stomach contents of approximately 10,000 fish representing 100 or more species, many of which live in close association with *Aplysia,* and have as yet found no evidence of any *Aplysia* remains. No other animal is known to prey on postmetamorphic *Aplysia.*

The probability of any more direct contribution of *Aplysia* to the food economy of the sea or to a prey-predator relationship seemed more removed by the demonstration of a toxin with cholinergic (acetylcholinelike) action from the digestive glands of the California sea hares (Winkler, 1961; Winkler, Tilton and Hardinge, in press). In addition to any effects of the strongly persistent, musky odor and of the protective secretions of these animals to be dealt with in an ensuing paper, this toxin would seem to make them a questionable article of predator diet, at least during the postmetamorphic adult stages.

While a specimen of the Solitary Great Green Anemone, *Anthopleura xanthogrammica* (Brandt), was being removed from the rocks in a lagoon at Lunada Bay, Palos Verdes, California (during mid-Nov), it regurgitated a young partially-digested specimen of *Aplysia californica* Cooper. Since these young sea hares were present in considerable numbers in the lagoon, other anemones were immediately examined. Five sea hare remains were obtained from the first six anemones checked. A further check of other anemones outside the lagoon, where fewer young sea hares were found, produced sea hare remains less frequently but doubled the number of *Aplysia* remains for study. One crab cheliped was the only other evidence of enteric contents recovered from the anemones.

This find showed an ecological relationship not previously reported and led to experiments to determine if the toxin-containing digestive gland was retained and digested, and, if retained, what the effect of the cholinergic activity of the *Aplysia* toxin (aplysin) might be on the anemone.

MATERIALS AND METHODS

All field studies were carried out and specimens were routinely obtained at Lunada Bay, Palos Verdes, California. Anemones were checked for enteric contents by inserting the index finger through the mouth, exploring the enteron therewith and extricating any solid matter found. Remains recovered were preserved in formol-alcohol. After preservation, remains were sketched, photographed, and weighed, and the digestive gland was carefully removed and reweighed separately.

In order to calculate the approximate original weight of a sea hare from the weight of its digestive gland, data obtained from previous observations were used (Winkler, 1961). A similar study was made with *A. vaccaria* Winkler.

The digestive glands of *A. vaccaria* and *A. californica* may readily be distinguished by their form and histology. The gland of the former is

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diffuse, composed of convoluted folds with large and numerous sulci interspersed; while the latter is compact, without folds or convolutions. The histology is distinguished by the appearance of large numbers of oval brown granules of approximately 100 μ in *A. californica,* while granules are only rarely found as sparse, irregular objects in an occasional specimen of *A. vaccaria.*

Studies on the intact animal were carried out both in the field and in laboratory–maintained specimens. Specimens were fed small living sea hares and pieces of digestive gland. Field specimens were injected with aplysin and the results noted.

Three types of isolated anemone muscle were used: (1) The septum retractor muscle, an internal vertical muscle near the medial margin of the septa; (2) The internal walls of the pharynx, removed and used in isolated baths; (3) Strips of the external circular muscle, 1×6 cm, cut from the body wall just below the tentacles. The latter specimens were cut from the living animals which were then replaced in the aquarium and soon recovered, regenerating the lost tissues.

The muscular tissue was ligated at both ends. One end was tied to a solid plastic support while the other activated a pressure transducer the output of which was fed through an amplifier into a Grass electronic recorder. The muscles were immersed in an aerated 30 ml sea water bath to which small amounts of the aplysin and acetylcholine were added. Aplysin was prepared as described by Winkler (1961) and Winkler, Tilton, and Hardinge (in press). Acetylcholine was used in 1% solution in all cases.

RESULTS

The condition of the partially digested remains of 10 sea hares (*Aplysia californica*) is shown on Figure 1, and the weights of the remains and those of the digestive glands with the calculated probable weights of the living animals are listed in Table 1. The per cent of total body weight of the digestive glands from 15 living *A. californica* were calculated in a previous experiment but are first reported herewith. The average of 10.8% (standard deviation ± 1.73%) is somewhat lower than that for *A. vaccaria* which averages 18% (standard deviation ± 3.83%). Since this present study utilized only preserved specimens on which no other data were available, all weights were calculated on the basis that the digestive gland represents 10% of the total weight. This figure may vary somewhat from fact but is probably quite close and seems to be adequate for the present study.

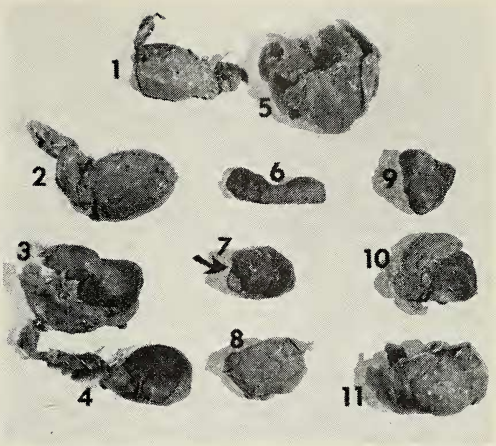

FIG. 1. Remains of *Aplysia californica* Cooper obtained from enteric cavities of the sea anemone, *Anthopleura xanthogrammica* (Brandt). Numbers correspond with those of analysis in Table 1.

In the field, experimental feeding of digestive gland to three anemones resulted in ingestion but in two cases expulsion took place during the limited period of observation. These experiments were supplemented by numerous feedings to four specimens maintained in an aerated, cooled salt water aquarium. The ingestion was immediate, followed by a slow regurgitation in 5–10 min. (Anemones were normally fed on frog muscle, which was not regurgitated). The regurgitated material was covered with an envelope of thick mucus.

Small sea hares were fed alive to anemones; and although ingested, in all except the case of a weakened animal (no. 1), the sea hares were able to escape. The fact remains, however, that in nature some at least do not escape.

The anemones in the field, when injected with aplysin, gave inconclusive results. The most uniform reaction was contraction of the area injected for a period of time, but it was difficult to interpret this as being greatly different from the effect of a simple puncture except that it was more persistent. When aplysin was released in the enteron the anemone would close in a few minutes, but part of the tentacles might maintain their expanded positions.

The isolated septum retractor muscle of the anemone was slightly contracted by aplysin, while the wall of the enteron (including the pharynx) was insensitive to large doses of both aplysin and acetylcholine. Regular jagged contractions were initiated in the former by acetylcholine but not by aplysin. These contractions disappeared upon washing and returned when acetylcholine was again added. The wall of the enteron and pharynx did not show a response when stimulated with glass rods inserted in the mouth of living sea anemones as long as the external oral margin was not touched. However, stimulation of the external oral margin yielded an unfailing response. Apparently the internal enteron wall is devoid of tactile receptors or ganglia producing contractatory responses.

Arcs of tissue taken from the external body wall and suspended in a tissue bath were very sensitive to acetylcholine and to aplysin. Strong responses were elicited which were of added interest in that, without the washing, the height of response fell off rapidly and returned to the base line or below it in 3–5 min (Fig. 2). Upon adding acetylcholine to such an aplysin-excited preparation, no renewed response could be elicited, even though the muscle had relaxed (Fig. 2*a*). The same was true when aplysin was added to an acetylcholine-excited strip (Fig. 2*b*). Washing did not immediately renew sensitivity. When aplysin was applied first, the washing time required before sensitivity returned was ½ hr or more, as compared to 2–6 min in the case of acetylcholine. These time periods reflect quite accurately the relative washing times required for aplysin and for acetylcholine in the frog rectus abdominis assay procedure of Chang and Gaddum (1933).

DISCUSSION AND CONCLUSIONS

The extent to which *Anthopleura xanthogrammica* was preying on young *A. californica* in this particular location is interesting relative to the position of *Aplysia* in the food chains of life in the sea.

The anemones, however, appear to be a nearly "dead-end street" as far as a food chain is concerned, since some species of anemones are known to have lived in captivity at least 70 years (Annandale, 1912; MacGinitie, 1949) and apparently are not greatly preyed upon. In addition, the predator in this case is of a lower order of life than the prey. Thus the enormous numbers of larvae produced (MacGinitie, 1934) would seem to be the principal contribution of *Aplysia* to the food cycles of the sea. According to this concept the adult sea hares serve largely as reproductive machines increasing the supply

TABLE 1

ANALYSIS OF REMAINS OF *A. californica* TAKEN FROM ENTERIC CAVITIES OF *Anthopleura xanthogrammica*

NUMBER*	SOURCE OF ANIMAL	WEIGHT IN GRAMS: Remains	Digestive Gland	Approximate Original Weight of Animal	Amount Consumed	% CONSUMED
1	fed animal	10	2	20	10	50
2	reef	21	10	100	79	79
3	animal	20	6	60	40	67
4	animal	13	5	50	37	74
5	reef	30	9	90	60	67
6	animal	3	1	10	7	70
7†	animal	7	4.5	45	38	85
8	animal	9	4	40	31	73
9	animal	7	3.5	35	28	79
10	animal	11	4	40	29	72
11	animal	25	8	80	55	69

* Number corresponds to those on photographs.
† The only specimen in which the taut translucent membrane surrounding the digestive gland had been penetrated.

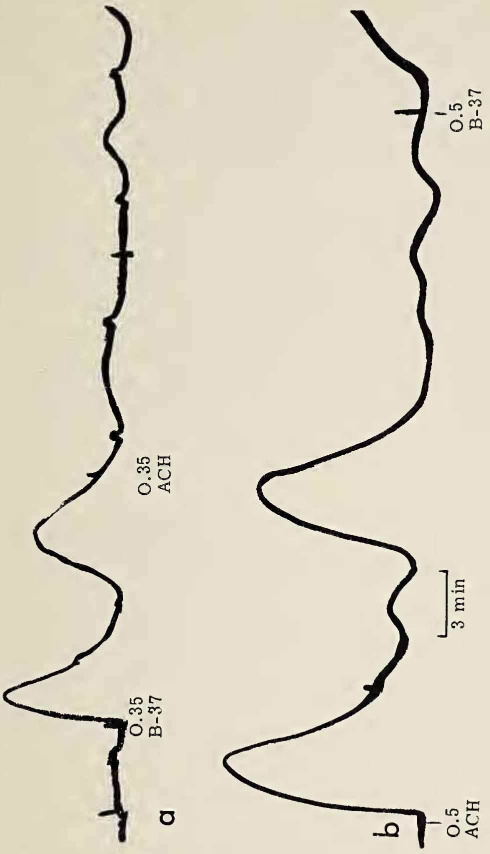

FIG. 2. Tracings of the reaction of strips of external body muscle of the anemone to aplysin (B-37) and to acetylcholine (ACh). It will be noted that aplysin, *a,* and acetylcholine, *b,* mutually block the action of the other, but that this has no depressatory effect on the irregular spontaneous contractions though both drugs often seem to stimulate their occurrence.

of microscopic organisms available as food. The findings of the present paper modify but little this latter concept.

Apparently the sea anemone consumes the sea hare without becoming a victim of the sea hare's digestive gland toxin because (1) the digestive gland appears usually to be regurgitated and (2) the toxin has only a transitory effect on the simple organ systems of the anemone. Study of the sea hares recovered from anemones suggested that the digestive gland is attacked by the digestive fluids of the anemone only after almost the entire sea hare has been consumed. The digestive gland of *A. californica* is consolidated and protected by a transparent membrane, which was found to be consistently exposed over a considerable area before it was finally penetrated. Thus most of the sea hare is digested and absorbed before sufficient irritation from the toxin is produced to stimulate rejection or to cause much effect on the anemone's system. The secretion of the thick mucus with which the sea hare digestive gland is coated by the anemone prior to its ejection is perhaps stimulated by local irritant qualities of the toxin, though Hyman (1940) states that this mucus is a normal reaction to any material ingested. The ejection itself is accomplished by a slow steady movement of the undesired material out of the mouth and over the surface of the perioral disk, at the margin of which it is finally released.

Aplysia toxin is a powerful cholinergic agent (Winkler, Tilton, and Hardinge, in press) acting upon both ganglia and parasympathetic nerve endings as well as on the neuromuscular junction in higher animals. The effect of acetylcholine on the external anemone muscle suggests that either acetylcholine or a similar agent is the transmitter substance. The spontaneous relaxation of anemone muscle experimentally contracted with either aplysin or acetylcholine is probably not the result of enzymatic destruction of the agent, as might occur with small amounts in higher animals, since no new contraction can be initiated by either acetylcholine or aplysin. Only after adequate washing can the muscles again respond.

The mutual antagonism would seem to indicate the same mechanism of action for aplysin as for acetylcholine. Certain cholinergic agents such as aplysin operate by mimicking the natural nerve transmitter substance, acetylcholine, but each has its own peculiar characteristics. In this case acetylcholine may be washed out in about 3 min, while aplysin takes ½ hr or more. The nerve receptors appear to be completely paralyzed and unable to be excited again until the transmitter substance has been completely removed.

According to Hyman (1940), the nervous system of anemones consists of two plexi: one, the epidermal plexus throughout the exterior body surface; the other, the gastrodermal plexus in the septa.

The epidermal plexus contains ganglion cells in the tentacles, oral disk, and pharynx. According to the Hertwigs, the ganglion cells are most numerous in the oral disc at the base of the tentacles where they form a centralized ring. Later workers have failed to verify this statement and Groselj (1909) locates the greatest concentration of the second nerve plexus in the upper end of the pharynx.

Thus considerable uncertainty as to the innervation of the sea anemones already exists—which may of course reflect species differences. These workers have probably correctly analyzed the species with which each worked. For the present species it seems obvious that the pharynx lacks nervous tissue sensitive to the tactile and chemical stimuli used, or that these agents initiate reactions other than contraction or relaxation (such as increased secretion of mucus or increased directional ciliary activity) which were not under study in the present paper. If these anemones are insensitive to acetylcholine it would then be likely that a chemical mediator other than acetylcholine may occur naturally, though neither epinephrine nor serotonin caused contraction of the pharynx in tissue bath.

The tactile sense is acute in the lips, which in this species consists of the scalloped margin of the mouth where it connects to the pharynx with an abrupt change of tissue color as well as of texture. The tactile sensitivity commences abruptly as a glass probe is passed onto the colored external tissue at the lips. This perioral disk area and the tentacles are very sensitive to tactile stimuli. These findings agree with the first innervation mentioned by Hyman (1940), as well as with her later discussion of the areas sensitive to tactile stimulation.

SUMMARY

1) Remains of *Aplysia californica* Cooper in various stages of digestion were taken from the enteron of various specimens of *Anthopleura xanthogrammica* (Brandt).

2) The estimated per cent of consumed material, in the case of 10 specimens taken from anemone digestive tracts, ran between 67 and 85%.

3) In no case was the digestive gland attacked by the digestive process.

4) In tissue baths aplysin and acetylcholine produced contractatory response of the external surface muscle, and no effect was noted on the internal wall of the enteron.

5) Tactile- or chemo-receptors, sensitive to acetylcholine and initiating contractatory responses, appear to be absent in the enteron and pharynx.

6) Segments of the external wall reacting completely to either acetylcholine or aplysin would relax to the starting level in 3–5 min. Subsequent application of the other agent showed the muscle to be unable to respond.

7) After washing, those treated initially with acetylcholine quickly regained their ability to respond. However, those treated initially with aplysin would respond again only after repeated washing.

8) It appears that the anemones may reject the digestive gland after having digested away the animal itself. However, if absorbed, aplysin would probably cause only a temporary contraction followed by relaxation and temporary paralysis.

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